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(54) Title: MULTIPLE SCLEROSIS T-CELL RECEPTOR

(57) Abstract

This invention is directed to therapeutic agents comprising peptides in turn comprising a portion of the T-cell manifested in multiple sclerosis. Embodiments of the invention include peptides, vaccines, receptors and entire (attenuated) T-cells. This invention is also directed to methods using the foregoing agents to suppress immune response against myelin basic protein, and/or to suppress T-cells that recognize an immunodominant epitope of human myelin basic protein. Additionally, this invention is directed to diagnostic methods and kits for diagnosing multiple sclerosis.

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MULTIPLE SCLEROSIS T-CELL RECEPTOR

The United States Government has rights to this invention by virtue of funding from Grant Nos. NS 24247, and NS 17182 from the National Institutes of Health.

This application is a continuation-in-part of PCT International Application No. U.S. 88/02139 filed June 24, 1988.

FIELD OF THE INVENTION

This invention pertains to agents and methods for treating Multiple Sclerosis. More specifically, the invention is directed to therapeutic agents comprising a T-cell receptor and fragments or analogs thereof which are believed to be involved in the pathogenic mechanism of the disease, and to methods of using such agents to suppress disease symptoms.

BACKGROUND OF THE INVENTION

Multiple Sclerosis (MS) is a chronic inflammatory disease of the central nervous system white matter of humans and is believed to be of autoimmune etiology. The disease is characterized by prominent T-cell and macrophage infiltrates, demyelination and neurological dysfunction. Myelin basic protein (MBP) has been extensively studied as a potential autoantigen in the disease because of its role as an inducing agent in the major animal model of MS, experimental allergic

encephalomyelitis (EAE), as well as its role in the human disease post viral encephalomyelitis.

A major hypothesis regarding the pathogenesis of MS is that T-cells reactive with myelin basic protein in the white matter of the CNS initiate the inflammatory process. The demonstration that activated T-cells specific for myelin basic protein (MBP) can be isolated from MS patients (Allegretta, M., et al., Science: 247: 778, 1990) implicates MBP-reactive T-cells in the pathogenesis of the disease.

Experimental allergic encephalomyelitis (EAE) is the primary animal model for MS. EAE can readily be induced in small mammals by immunization with myelin basic protein (MBP) in an appropriate adjuvant or by passive transfer of CD4+, MBP-reactive T-cells (Alvord Jr, E.C., et al. eds. in Experimental Allergic Encephalomyelitis: A Useful Model for Multiple Sclerosis, A. R. Liss, N.Y., 1984; Makhtarian, D.E., et al. Nature 309: 356, 1984; Ben-Nun, A. et al. J. Immunol. 129:303, 1982). The T-cells that induce EAE in both mice and rats recognize specifically peptides corresponding to species-specific immunodominant regions of MBP presented on antigen-presenting cells by unique Major Histocompatibility Complex (MHC) class II molecules.

T-cell receptors are composed of two distinct chains of protein material. Certain T-cell receptors (TCRs), composed of V-beta (VB) chains and V-alpha (VA) chains, are known to recognize MBP. In SJL/PL mice, encephalitogenic (i.e., disease-inducing when administered to mice) T-cells having these receptors recognize an N-terminal mouse MBP peptide (residues 1-9) presented by an MHC molecule (Zamvil, S.S. et al., Nature 324: 258, 1986) encoded by the mouse gene H-2. The majority of T-cell receptors recognizing this peptide presented in connection with the MHC are encoded by the mouse TCR genes VB8.2 and VA2 or VA4. In Lewis rats, TCR gene segments that are homologous with the mouse VB8.2 and TCR VA2 genes have been found in encephalitogenic T-cells which recognize MBP residues 68-88 in the context of the Lewis rat MHC (Burns, F.R., et al., J. Exp. Med. 169: 27, 1989). Administration of a VB8.2-

specific monoclonal antibody (i.e., an antibody recognizing the product VB8.2 expressed by the corresponding gene) to mice has been shown to be effective in treating murine EAE. Immunization with peptides specifically corresponding to the TCR VB8.2 amino acid sequence ameliorates EAE in the Lewis rat (Vandenbark, A.A., et al., Nature 341: 541-544, 1989; Howell, M.D. et al., Science: 246, 668; 1989). However, the regions of an autoantigen (such as MBP) that behave as immunodominant regions are species specific. It has not heretofore been determined if common V-gene usage in TCR V-genes exists in humans among T-cells recognizing immunodominant regions of MBP nor have these immunodominant regions been positively identified in MS patients.

The current treatments for MS involve administration of drugs which act in a non-specific fashion to suppress the immune response in the subject. Examples of such drugs are cyclophosphamide, Imuran (azathioprine) and the cyclosporin A. Steroid compounds such as prednisone and methylprednisolone are also employed in many instances. These drugs have limited efficacy against MS. Use of such drugs is limited by toxicity and by the fact that they induce "global" immunosuppression upon prolonged treatment, i.e., they down regulate the normal protective immune response to pathogenic microorganisms thereby increasing the risk of infection. A further drawback is the increased risk that malignancies will develop in patients receiving prolonged global immunosuppression.

Other therapies are being developed for the treatment of autoimmune diseases in general and MS in particular. U.S. Patent Application Serial No. 65,794 filed June 24, 1987 (now abandoned) and copending International Patent Application PCT/US88/02139, filed June 24, 1988, disclose that oral or enteral administration of myelin basic protein and of disease inducing and non-inducing fragments and analogs thereof is effective in suppressing acute monophasic EAE and are useful in suppressing MS symptoms when similarly administered.

U.S. Patent Application Serial No. 454,806 filed December 20, 1989 discloses the aerosol administration of

autoantigens, disease-suppressive fragments of said autoantigens and analogs thereof as an effective treatment for treating T-cell mediated autoimmune diseases such as MS.

A U.S. patent application filed March 3, 1990 entitled "Enhancement of the Down Regulation of Autoimmune Diseases by Oral Administration of Autoantigens" discloses synergists (enhancers) for use with oral administration of autoantigens, disease-suppressive fragments and analogs thereof as effective treatments for T-cell mediated autoimmune diseases.

In furtherance of the efforts and goals expressed in these prior applications, i.e., the design of effective, specific therapeutic treatments for MS, what is needed in the art is to determine if common V-gene usage associated with Major Histocompatibility Complex antigens exists in humans suffering from MS. In other words, there is a need to determine whether encephalitogenic T-cells isolated from human MS victims use restricted TCR VB genes as observed in rodents and whether this function can be exploited to combat disease symptoms. In addition, it is necessary to determine the major immunodominant epitope domain present on human MBP, again with a view towards exploiting all or part of such domain towards therapeutic ends.

It is an object of the present invention to provide agents and methods based upon the human TCR for treating humans suffering from autoimmune diseases having the symptoms of MS.

Another object of the present invention is to provide compositions and pharmaceutical formulations useful for treating humans suffering from autoimmune diseases having the symptoms of MS.

A still further object of the invention is to provide compositions and pharmaceutical formulations useful for administration to humans for the purpose of preventing or attenuating to the manifestation (i.e., clinical symptoms) of autoimmune diseases having the symptoms of MS. Another object of this invention is to provide reagents useful in diagnosis of MS (or of another disease presenting with the same symptoms). (For example TCR-based peptides or peptides based on the im-

munodominant domain of the human MBP can constitute such diagnostic reagents.)

These and other objects of the present invention will be apparent to those of ordinary skill in the art in light of the present specification, drawings and claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is an autoradiograph showing PCR amplification of cDNA's from 18 T-cell lines which were generated from five MS patients and which were reactive with MBP residues 84-102.

Figure 2 is an autoradiograph of a Southern blot analysis of TCR VB and JB gene usage for MBP-reactive T-cell lines generated from peripheral blood of an MS patient.

Figure 3 is a bar graph showing the frequency of MBP reactive T-cells to different human MBP peptides isolated from MS patients and controls.

Figure 4 is a series of bar graphs showing the reactivity of T-cells isolated from MS patients and controls to different regions of the human MBP polypeptide in relationship to whether these patients have certain MHC antigens.

SUMMARY OF THE INVENTION

This invention is directed to therapeutic agents comprising peptides in turn comprising a portion of the T-cell receptor for an antigen involved in immune response of the type manifested in multiple sclerosis. Embodiments of the invention include peptides, vaccines, receptors and entire (attenuated) T-cells.

This invention is also directed to methods using the foregoing agents to suppress immune response against myelin basic protein, and/or to suppress T-cells that recognize an immunodominant epitope of human myelin basic protein.

Additionally, this invention is directed to diagnostic methods and kits for diagnosing multiple sclerosis.

DETAILED DESCRIPTION OF THE INVENTION

All patent applications, patents and literature cited in this specification are hereby incorporated by reference in their entirety.

As used herein, "treatment" is meant to include both prophylactic treatment to prevent an autoimmune disease having the symptoms of MS (or the manifestation of clinical symptoms thereof) as well as the therapeutic treatment, i.e. the suppression or any measurable alleviation of one or more symptoms after the onset of a disease presenting the symptoms of MS.

The term "autoantigen" is defined as any substance normally found within a mammal that, in an abnormal situation, is no longer recognized as part of the mammal itself by the lymphocytes or antibodies of that mammal, and is therefore attacked by the immunoregulatory system as though it were a foreign substance. Examples are MBP and proteolipid peptide (PLP).

"Immunodominant epitope" of an autoantigen (such as MBP) means an antigenic determinant recognized by a majority (although not necessarily an absolute majority) of T-cells of a sensitive species to which such T-cells will mount or help mount an immune response.

"Immunodominant regions" or "immunodominant domains" of an autoantigen (MBP) are defined herein as those regions of the autoantigen containing an immunodominant epitope. The structures (and/or location within the MBP molecule) of immunodominant epitopes (and regions) of MBP vary between species, and are, therefore, species-specific.

"Autoimmune suppressive agents" are defined herein as peptides having the amino acid sequences of (or contained in) VB17 and/or VB12 of the T-cell receptor or analogs thereof as well as other agents (such as attenuated VB17- or VB12-containing T-cells), which when administered to a mammal suffering from a disease having the symptoms of MS will suppress one or more of such symptoms. (The minimum sequence length of the active peptides of the present invention is about 20 amino acids. There is no particular maximum as long as activity is

preserved. For example, the entire TCR or even entire T-cells could be used.)

"MHC" or "Major Histocompatibility Complex" is defined as a complex series of mammalian cell surface proteins present on the surface of activated T-cells, macrophages and other immune system cells. The MHC plays a central role in many aspects of immunity both in presenting histocompatibility (or transplantation) antigens and in regulating the immune response against conventional (foreign) antigens. There are two types of MHC protein molecules, class I and class II. The human MHC genes are located on human chromosome 6 and the mouse MHC genes are located in the H-2 genetic locus on mouse chromosome 17.

"Class II MHC molecules" are membrane glycoproteins that form part of the MHC. Class II MHC molecules are found mainly on cells of the immune system including B-cells, macrophages, brain astrocytes, epidermal Langerhan's cells, dendritic cells, thymic epithelium and helper T-cells. Class II MHC molecules are involved in regulating the immune response during tissue graft rejection, stimulation of antibody production, graft-versus-host reactions and in the recognition of "self" (or autologous) antigens, among other phenomena. In the specification below, MHC shall be used interchangeably with "Class II MHC". The MHC genes will be referred to as "MHC genes".

As used herein, "T-cells" or "T-lymphocytes" are defined as immune system cells, derived from stem cells located within hematopoietic (i.e. blood forming) tissues. There are three broad categories of T-cells: Helper, Suppressor and Cytotoxic. T-cells express either the CD4 antigen (and are then called CD4+ T-cells) or the CD8 antigen (in which case they are called CD8+ T-cells) on their cell surface. The expression of CD4 or CD8 antigens by peripheral (circulating) T-cells correlates with the function and specificity of the T-cell. "Helper T-cells" which are CD4+ recognized antigens and Class II MHC molecules and perform helper or regulatory functions. "Cytotoxic" and "Suppressor" T-cells (which are CD8+)

recognize antigens and Class I MHC molecules perform suppressor and cytotoxic functions.

"T-cell receptor" or "TCR" is defined herein as the antigen recognition receptor present on the surface of T-cells. TCR is, therefore, the receptor that binds a molecule which the immune system recognizes -- and presents -- as an antigen (whether the molecule is foreign or autologous, the latter being the case in an autoimmune disease). A majority of T-cells express a TCR composed of a disulfide-bonded heterodimer protein containing one alpha (A) and one beta (B) chain whereas a minority of T-cells express two different chains (gamma and delta). The TCR is composed of an A and a B chain, each of which comprises a variable and a constant region. (Tilinghast, J.P. et al., Science 233: 879, 1986; Concannon, P. et al., Proc. Natl. Acad Sci USA 83: 6589, 1986, Kimura, N. et al., J. Exp. Med. 164: 739, 1986; Toyonaga, B. et al., Proc Natl. Acad. Sci USA 82: 8624, 1985.) The variable region in turn comprises a "variable", a "diversity" and "joining" segment. The junction among the variable, diversity and joining segment is postulated to be the site of antigen recognition by T-cells.

T-cells initiate the immune response when antigen presenting cells (APC), such as mononuclear phagocytes (macrophages, monocytes), Langerhan's cells and follicular dendritic cells, initially take up, process (digest) and present antigenic fragments of the polypeptide on their cell surface (in connection with their MHC). CD4+ T-cells recognize antigen molecules exclusively when the protein is processed and peptide fragments thereof are presented by APCs that express Class II MHC molecules.

T-cell recognition of an antigen reflects a trimolecular interaction between the TCR, MHC molecules and peptides processed by APCs via a cleft or pocket in the three-dimensional structure of the Class II MHC molecule. (Bjorkman, P.J., et al., 1987, Nature, 329:506 and 329:512).

The present inventors have identified an immunodominant region of MBP resident within a portion of the MBP amino acid sequence (residues 82-104) and two T-cell receptor gene

segments which correspond to VB17 and VB12. As shown in Example 2 below, the present inventors have identified human MBP amino acid residues 84-102 as the basis of an immunodominant domain of MBP recognized by a majority of peripheral T-cells isolated from patients suffering from MS. In addition, the present inventors have determined that T-cells reacting with the immunodominant epitope of MBP often also posses the MHC Class II haplotype DR2 gene. The corresponding MHC antigen of such T-cells binds MBP within immunodominant domain composed of residues 82-104 in association with the DR2 phenotype. Since DR2 is most common in patients with MS, these cells can be isolated, identified and used not only to diagnose but also to treat patients with MS (as will be explained below).

In the animal model (EAE) T-cell receptors comprising a portion of the animal VB8.2 sequence have been used to treat the disease and shown to act by eliminating disease-inducing T-cells. In particular, in the animal model, peptides comprising the sequences Thr-Leu-Cys-Ala-Ser-Ser and Thr-Leu-Cys-Ala-Ser-Arg which may correspond to exposed (surface) portions of mouse and rat VB8.2 have been determined (in mouse and rat models) to combat the autoimmune disease model by eliminating Helper T-cells.

The present invention can be advantageously used in the design of specific therapeutic agents useful for treating a human suffering from a disease with the symptoms of MS. For example, as shown in Examples 1 and 2 below, peptides comprising sequences of the human VB17 and VB12 can be constructed, e.g., the amino acid sequences Asp Thr Asp Lys Gly Glu Val Tyr Asp Gly (from VB12) and Phe Gln Lys Gly Asp Ile Ala Glu Gly Tyr (from VB17) and used for therapeutic purposes. Other active peptides are those comprising the sequence JYYSQIVNDFQKGDIAEGYS. Additional active peptides can be designed based on the amino acid sequences of the entire human VB17 and VB12 or fragments or analogs thereof.

The amino acid sequence for human VB12 (and one of the possible nucleic acids encoding it) is set forth below:

LeuArgCysHisGlnThrGluAsnHisArgTyrMetTyrArgGlnAspProGlyHisGly
CTGAGATGTCACCAGACTGAGAACCACCGCTATATGTACCGACAAGACCCGGGGCATGGG

LeuArgLeuIleHisTyrSerTyrGlyValLysAspThrAspLysGlyGluValSerAsp
CTGAGGCTGATCCATTACTCATATGGTGTTAAAGATACTGACAAAGGAGAAGTCTCAGAT

GlyTrySerValSerArgSerLysThrGluAspPheLeuLeuThrLeuGluSerAlaThr
GGCTATAGTGTCTCTAGATCAAAGACAGAGGATTTCCTCCTCACTCTGGAGTCCGCTACC

SerSerGlnThrSerValTyrPheCysAlaAsn
AGCTCCCAGACATCTGTGTACTTCTGTGCCAAT

The amino acid sequence for human VB17 (and one of the possible nucleic acids encoding it) is set forth below:

AspGlyGlyIleThrGlnSerProLysTyrLeuPheArgLysGluGlyGlnAsnValThr
GATGGTGGAATCACTCAGTCCCCAAAGTACCTGTTCAGAAAGGAAGGACAGAATGTGACC

LeuSerCysGluGlnAsnLeuAsnHisAspAlaMetTyrTrpTyrArgGlnAspProGly
CTGAGTTGTGAACAGAATTTGAACCACGATGCCATGTACTGGTACCGACAGGACCCAGGG

GlnGlyLeuArgLeuIleTyrTyrSerGlnIleValAsnAspPheGlnLysGlyAspIle
CAAGGGCTGAGATTGATCTACTACTCACAGATAGTAAATGACTTTCAGAAAGGAGATATA

AlaGluGlyTrySerValSerArgGluLysLysGluSerPheProLeuThrValThrSer
GCTGAAGGGTACAGCGTCTCTCGGGAGAAGAAGGAATCCTTTCCTCTCACTGTGACATCG

AlaGlnLysAsnProThrAlaPheTyrLeuCysAlaSerSer
GCCCAAAAGAACCCGACAGCTTTCTATCTCTGTGCCAGTAGT

(Notwithstanding the identity between mouse peptide TKCASS and the C-terminal human VB17, peptides encompassing this amino acid sequence are not expected to be active in humans because this sequence is a fairly common terminal sequence in human VB chains and hence would not have as specialized a function as required for activity in suppressing MS symptoms.)

Without wishing to be bound by theory, it is believed that administration of VB17- or VB12-based peptides especially those incorporating the fragments previously identified above or active analogs thereof to patients suffering from MS will block the TCR or kill T-cells that express TCR and thereby block the induction or activation of Helper T-cells involved in mounting an immune response against the myelin sheath of the central nervous system (CNS) in patients suffering from MS. The mechanism of this may involve the production of anti-VB (12 or 17) antibodies, i.e., native antibodies that will recognize

VB12 and/or VB17 and therefore bind to TCR. Whatever the mechanism of action, these VB12- and VB17-based peptides are fully expected to be effective in attenuating or eliminating symptoms of MS or of a disease presenting with the same symptoms and are, therefore, expected to be useful therapeutic agents or adjuncts to MS therapy. (For example, parenteral administration of such peptides may supplement or be supplemented by oral and/or aerosol administration of MBP or fragments or analogs of MBP as disclosed for example in U.S. patent appls. Serial No. 487,732 filed March 2, 1990 and entitled "Enhancemen of the Down-Regulation of Autoimmune Diseases by Oral Administration of Autoantigens", and Serial No. 454,806 filed December 20, 1989.

In addition, healthy individuals susceptible to MS (i.e. individuals having the DR2 haplotype) and expressing VB17 or VB12 TCR on their T-cells, and therefore having T-cells that proliferate (or that could be induced to proliferate) in response to the presentation of the immunodominant region of human MBP may also benefit from prophylactic administration of the peptide. In Example 2 below, VB17 was significantly less frequently present (only in approximately 9.4% of the cell population collected) on T-cells isolated from a normal individual than was present on the T-cell lines reactive with human MBP amino acid residues 84-102 (53.9%) isolated from five MS patients. In addition, VB12 was identified on 35% (7/20) of T-cell lines reactive with MBP amino acid residues 84-102 isolated from 4 MS patients (as opposed to 15% of T-cells from normal controls). These results show that the VB17 and VB12 TCR peptides are selectively involved in the recognition of the immunodominant (possibly encephalitogenic) human MBP region. Therefore, it is anticipated that these peptides or fragments or analogs thereof will provide safe, effective therapeutic agents for treatment or prophylaxis of humans against MS symptoms.

Peptides based on the sequences of VB17 or VB12 for use in the present invention can be synthesized using well-known solid phase methods (Merrifield, R.B. Fed. Proc. Am. Soc. Ex.

Biol. 21: 412, 1962 and J. Am. Chem. Soc. 85: 2149, 1963; Mitchel, A.R. et al, J. Am. Chem. Soc. 98: 7357, 1976; Tam, J. et al., J. Am. Chem. Soc. 105: 6442, 1983). Alternatively, such peptides can be synthesized by recombinant DNA techniques, as is now well-known in the art (Maniatis et al. Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratories, NY, 1982, see pp. 51-54 and pp. 412-30). For example, these peptides can be obtained as the expression products after incorporation of DNA sequences encoding VB12 or VB17 (or fragments or analogs thereof) into expression vectors and introduction of such vectors into suitable eukaryotic or prokaryotic hosts that will express the desired peptides individually or as part of fusion peptides or proteins.

Peptide analogs can be designed using the known amino acid sequences encoded by the VB17 or VB12 genes as disclosed below, using the synthetic or recombinant techniques described above and the methods of, e.g., Eyler, E.H., in Advances in Experimental Medicine and Biology 98: 259-281, 1978. For example, a peptide having a sequence based upon the amino acid sequence of VB12 or VB17 can be chemically synthesized using the above-described techniques. The peptide can be tested for disease-suppressive activity when administered to a mammal using, for example, the experimental protocol of Howell, M.D. et al., Science, 246: 668, 1989 or Vanderbark, A.A. et al., Nahire, 341: 541, 1989.

In addition, T-cells which are VB17+ or VB12+ can be isolated from patients suffering from MS and identified using the techniques described in Examples 1 and 2 below. These isolated VB17+ or VB12+ T-cells can be expanded, cloned, attenuated (as described by Lider, O., et al., 1986, Ann. N.Y. Acad. Sci., pp.267-273 and by Weiner, H.L., et al. (Abstr.) Neurology (Suppl. 1) 69:172, 1989) and used as specific therapeutic agents/immunogens to treat patients suffering from MS (preferably the same patents from whom the T-cells were originally isolated). T-cell attenuation can be effected, for example, by exposing the T-cells to 0.1% glutaraldehyde for 15 min. at room temperature. T-cell clones grown to 50 million

cells in <u>vitro</u> and attenuated (as described) can be stored in phosphate buffer saline (PBS). A dosage of 50 million thus treated VB17+ and/or VB12+ cells can be injected e.g. subcutaneously.

The present invention also provides a kit containing isolated nucleic acids (RNA or DNA) having the sequence 5'GATACTGACAAAGGAGAAGTCTCAGATGGC3' and/or 5'TTTCAGAAAG-GAGATATAGCTGAAGGGTAC3' or analogs thereof encoding all or portion of VB12 and VB17, respectively, and sequences which hybridize with these sequences under stringent hybridization conditions (such as those described below in Example 1) can be used to diagnose MS and susceptibility to MS, and to monitor disease progression. T-cells can be isolated from patients, cloned, expanded and probed for the presence of VB12 and/or VB17 using for example the techniques described below in Examples 1 and 2, or other assay techniques well-known in the art.

The present invention also provides pharmaceutical formulations and dosage forms for use in treating mammals suffering from diseases having the symptoms of MS. In general such dosage forms contain one or more autoimmune-disease suppressive agents comprising peptides in turn comprising (i) the sequence of human VB12 and/or VB17 and (ii) disease suppressive fragments and analogs thereof, in an amount effective to treat or prevent one or more clinical symptoms of MS. Any clinically significant attenuation of one or more symptoms of MS that has been treated pursuant to the methods of the present invention is considered to be a treatment of such disease within the scope of the invention.

The autoimmune disease suppressive agents of the present invention may also encompass additional amino acids in sequences leading or following the VB17 or VB12- based sequences as long as these additional sequences do not defeat the disease-suppressive function of such agents. Testing of such constructs for disease-suppressive activity can be easily done using, for example, one or more of the methods described below.

It will be appreciated that the unit content of active ingredient or ingredients contained in an individual dose of each dosage form need not in itself constitute an effective amount for treating MS since the necessary effective amount can be reached by administration of a plurality of dosage units.

The pharmaceutical formulations of the present invention may include, as optional ingredients, pharmaceutically acceptable vehicles, carriers, diluents, solubilizing or emulsifying agents, and salts of the type that are well-known in the art. Nonlimiting examples of such substances include 0.5N saline in distilled water for parenteral use, lyophilized T-cell receptor or peptide diluted in lactose for oral use, adjuvants such as alum or tenanus toxoid or MAPS (disclosed in J.R. Tam et al., J. Exp. Med. 171:299-306, 1990; and Tam, J.R. Proc. Natl. Acad. Sci. 85:5409, 1988) for vaccination with peptides although larger peptide constructs such as whole TCR may not need an adjuvant.

The preferred route of administration of the suppressive agents of the present invention is in a parenteral form including intraperitoneal, intravenous, intradermal and most preferably subcutaneous administration routes. Preferred pharmaceutical formulations may comprise for example, formulations containing between about 0.3 mg and about 200 mg of one or more of the agents of the present invention specific for MS.

In general, the VB12- or VB17-based peptide or analog is introduced to a mammal in an amount preferably ranging between about 0.3 mg per kg body weight of said mammal and about 200 mg per kg body weight of said mammal preferably administered once every 3 months and may be administered in a single dosage form or multiple dosage forms. The exact amount and frequency of administration to a patient is subject to optimization and may vary depending on the stage, frequency of manifestation and severity of the patient's disease and the physical condition of the patient, as is well known in the art. Such optimization is preferably effected on a case-by-case basis. Customized therapy is common for MS patients and thus, optimization of dosage represents ordinary experimentation.

One preferred method of optimizing dosage is as follows: T-cells are obtained from a patient and cultured, the culture is expanded and T-cell DNA is collected.

The techniques described below in Examples 1-3 can be used to monitor the effectiveness of the methods of the present invention and optimize the amount and frequency of administration of the disease suppressive agents of the present invention.

T-cells can be isolated from a patient's peripheral blood, amplified and cloned as described in Examples 1-3 below (before and/or after treatment according to the present invention) and probed for the presence of VB12+ and/or VB17+ T-cells using PCR amplification with the specific VB primers shown in Table 2 below. A reduction or elimination of VB12+ or VB17+ T-cells after treatment with VB12 and/or VB17 based peptides of the present invention will provide an objective measurement of a patient's disease status. Therefore, the exact amount and frequency of administration of the agents of the present invention can be optimized.

Alternatively, antibodies (either polyclonal or monclonal) can be obtained directed against the VB12 and/or VB17 polypeptides of the present invention (using conventional techniques well known and used in the art) to assay for the presence of VB12+ and/or VB17+ T-cells in a patient's peripheral blood before and/or after treatment according to the present invention.

When isolated, attenuated VB17+ or VB12+ T-cells are administered to a patient, either prophylactically or for the treatment of active disease, the effective amounts can be easily determined, for example as follows: An amount of such T-cells, e.g., 50 million, is administered to a patient. Two weeks later, T-cells are collected from the patient and probed for the presence of T-cells expressing VB12 or VB17. If such T-cells have been significantly reduced, the dosage is effective. The preferred route of administration for this embodiment of the present invention is parenteral, and most preferably subcutaneous.

As shown in the Examples below, reactivity with MBP residues 84-102 is associated with the DR2 gene. The TCR VB gene used in three healthy DR2+ individuals was examined (controls 1-3, Table 3). 5/5 cell lines from a normal DR2+ subject were VB17+, whereas one of the two cell lines from another normal DR2+ was VB12+. These data show that VB17 and VB12 are TCR recognition elements for this immunodominant region in MS patients and in healthy DR2+ individuals.

The present invention is described further below in specific examples which are intended to illustrate the present invention without limiting its scope.

EXAMPLE 1: TECHNIQUES

MBP was extracted from human brain tissue and purified on a CM-52 column using the highest molecular weight peak (18kD) as described (Chou, F.C.-H. et al J. Biol. Chem. 251: 2671, 1976). MBP peptides were synthesized using a solid phase method and were obtained from a commercial laboratory (Bio-search Lab Inc., San Raphael, CA) and were purified by high pressure liquid chromatography. The MBP peptide fragments used are set forth below in Table 1.

TABLE 1

MBP Amino Acid Residues	Sequence	MBP Amino Acid Residues	Sequence
1-20:	ASQKRPSQRHGSKYLATAST	11-30:	GSKYLATASTMDHARHGFLP
21-40:	MDHARHGFLPRHRDTGILDS	31-50:	RHRDTGILDSIGRFFGGDRG
41-60:	IGRFFGGDRGAPKRGSGKDS	51-70:	APKRGSGKDSHHPARTAHYG
61-82:	HHPARTAHYGSLPQKSHGRT	71-92:	SLPQKSHGRTQDENPVVHFF
84-102:	DENPVVHFFKNIVTPRTPP	93-112:	KNIVTPRTPPPSQGKGRGLS
113-132:	LSRFSWGAEGQRPGFGYGGR	124-142:	RPGFGYGGRASDYKSAHKG
143-168:	FKGVDAQGTLSKIFKLGGRD		

T-cell receptor TCR VB gene usage was determined by polymerase chain reaction (PCR) amplification using a panel of TCR VB primers followed by Southern blotting. T-cell lines were established from peripheral blood mononuclear cells by two rounds of stimulation with MBP followed by stimulation with an immunodominant human MBP peptide (amino acid residues 84-102), immunodominance of which had been determined by proliferation assays (as described in Example 2) using the Table 1 panel of 13 overlapping MBP peptides. Following a third round of stimulation with their specific MBP peptide, RNA was extracted from MBP-reactive T-cell culture pellets (20,000-50,000 cells) by extraction with guanidium-isothiocyanate/phenol-chloroform and isopropanol precipitation in the presence of carrier tRNA. Single-stranded cDNAs were synthesized using oligo-dT and AMV-reverse transcriptase (both available commercially from Bethesda Research Laboratories, Gaithersburg, MD). PCR (polymerase chain reaction as disclosed in U.S. Patent Nos.

4,800,159 issued January 24, 1989; 4,683195 issued July 28, 1987; and 4,683,202 issued July 28, 1987) amplification was performed using a panel of 19 oligonucleotides (specific for published TCR VB families -- VB 1-20, Table 2) corresponding to the CDR2 region of the TCR B-chain and a CB (constant region of B chain) primer (Table 2) (as disclosed in Tilinghast, J.P. et al., Science 233: 879, 1986; Concannon, P. et al., Proc. Natl. Acad. Sci. 83: 6598, 1986; Kimura, N. et al., J. Exp. Med. 164: 739, 1986; Toyonaga, B. et al. Proc. Natl. Acad. Sci. 82: 8624, 1985; Kimura, N. et al., Eur. J. Immunol. 17: 375, 1987). Amplifications were done for thirty cycles (94°C 1 min., 55°C 2 min., 72°C 3 min.) using 1 microgram of each primer in 50 microliter reactions. Amplified products were separated in 1% agarose gels, transferred to nitrocellulose and Southern blots were hybridized with an internal oligonucleotide TCR-CB probe (Table 2). Probes were endlabeled with ^{32}P gamma-ATP and T4 polynucleotide kinase (Bethesda Research Labs.) to a specific activity of 10^8cpm/ug and hybridized in 6xSSC/5xDen-hardt's/0.05% pyrophosphate/100ug/ml denatured DNA/0.5% SDS for 18 hours at 37°C. Blots were washed at a final stringency of 6xSSC/70°C and autoradiographed for 2-18 hours. T-cell lines that were positive for more than two VB segments were considered not to be derived from a single MBP reactive T-cell and therefore excluded from analysis.

For sequencing, amplifications of cDNAs were performed with a VB17 primer (Table 2) specific for the leader segment containing an internal Pst I restriction site. Amplified DNA was treated with proteinase K, phenol/chloroform extracted, ethanol precipitated and digested with restriction endonucleases Bgl II and Pst I (available commercially, e.g., from Bethesda Research Labs., supra). Gel-purified DNA was ligated into M13 mp18 and single-stranded DNA was sequenced by the dideoxy-method (Sanger, F., et al., 1977, Proc. Nat'l. Acad. Sci., 74:5463). Negative controls were included during the procedure to test for possible contamination of RNA samples or reagents used for cDNA synthesis and amplification. The VB,

CB and JB2.1 primer sequences used are set forth below in Table 2.

Amplified and non-amplified samples were handled separately, reagents were aliquoted and tested for the presence of amplified material and negative controls were included for different experimental steps (RNA isolation, cDNA synthesis, PCR amplification).

TABLE 2

VB1	5'AAGAGAGAGCAAAAGGAAACATTCTTGAAC3'
VB2	5'GCTCCAAGGCCACATACGAGCAAGGCGTCG3'
VB3	5'AAAATGAAAGAAAAAGGAGATATTCCTGAG3'
VB4	5'CTGAGGCCACATATGAGAGTGGATTTGTCA3'
VB5	5'CAGAGAAACAAAGGAAACTTCCCTGGTCGA3'
VB6	5'GGGTGCGGCAGATGACTCAGGGCTGCCCAA3'
VB7	5'ATAAATGAAAGTGTGCCAAGTCGCTTCTCA3'
VB8	5'AACGTTCCGATAGATGATTCAGGGATGCCC3'
VB9	5'CATTATAAATGAAACAGTTCCAAATCGCTT3'
VB10	5'CTTATTCAGAAAGCAGAAATAATCAATGAG3'
VB11	5'TCCACAGAGAAGGGAGATCTTTCCTCTGAG3'
VB12	5'GATACTGACAAAGGAGAAGTCTCAGATGGC3'
VB14	5'GTGACTGATAAGGGAGATGTTCCTGAAGGG3'
VB15	5'GATATAAACAAAGGAGAGATCTCTGATGGA3'
VB16	5'CATGATAATCTTTATCGACGTGTTATGGGA3'
VB17	5'TTTCAGAAAGGAGATATAGCTGAAGGGTAC3'
VB18	5'GATGAGTCAGGAATGCCAAAGGAACGATTT3'
VB19	5'CAAGAAACGGAGATGCACAAGAAGCGATTC3'
VB20	5'ACCGACAGGCTGCAGGCAGGGGCCTCCAGC3'
CB	5'GGCAGACAGGACCCTTGCTGGTAGGACAC3'
C-probe	5'TTCTGATGGCTCAAACACAGCGACCTCGGG3'
VB17-Leader	5'AGCAACCAGGTGCTCTGCAGTGTGGTCCTT3'
JB2.1	5'CCCTGGCCCGAAGAACTGCTCATTGTAGGA3'

EXAMPLE 2: IDENTIFICATION OF VB GENE USAGE IN T-CELLS ISOLATED FROM MS PATIENTS

Two series of experiments were performed to test the validity of the above-described approach. First, it was demonstrated that all Table 2 primers except VB20 were able to

amplify cDNA from peripheral blood T-cells (Figure 1). Secondly, the specificity of PCR amplifications was examined by analysis of VB gene usage in 69 independent T-cell clones previously established by single cell cloning with mitogen (such as phytohemagglutin, -- "PHA" -- and interleukin-2). Due to the high cloning efficiencies obtained, these clones provided a representative analysis of VB gene usage among peripheral blood T-cells. TCR VB gene usage could be determined for 65/69 (94.2%) of these T-cell clones indicating that a large proportion of the TCR VB repertoire was covered by the VB primers. While 58 of these clones (84%) were positive for a single VB, 7 clones (10.1%) were double-positive, possibly due to the presence of two rearranged and expressed TCR VB genes.

The TCR VB gene usage was then analyzed in sixty-five MBP-specific T-cell lines established from five patients with clinically-defined relapsing-remitting MS. Representative Southern blots from MBP reactive T-cell lines are shown in Figure 1 and VB genes usage for all cell lines analyzed are set forth in Table 3 below.

TABLE 3

MBP PEPTIDE 84-102 REACTIVE T-CELL LINES

MULTIPLE SCLEROSIS

CELL LINE	TCR VB	CELL LINE	TCR VB	CELL LINE	TCR VB
Patient 1 (DR2.DR7)		HY.2C12	VB17,VB1	Cy.2C2	VB12
HY.1B12	VB17	Hy.2E2	VB17,VB1	Cy.3F6	VB12
Hy.1G9	VB17	Hy.2E11	VB17,VB2	Cy.4C1	VB12
Hy.1H7	VB17	Hy.3A11	VB17,VB2	Patient 3 (DR2,DR4)	
Hy.2C9	VB17	Hy.2C8	VB17,VB11	Ns.2A5	VB1
Hy.2E4	VB17	Hy.3B7	VB4	Ns.2C10	VB3,VB14
Hy.2E6	VB17	Hy.3C3	VB4	Ns.2D11	VB5,VB7
Hy.2F10	VB17	Hy.3C6	VB4	Ns.1G11	VB12,VB17
Hy.2G5	VB17	Hy.2F11	VB7	Ns.2E2	VB12,VB17
Hy.2G11	VB17	Hy.3B12	VB7	Patient 4 (DR2,DR7)	
Hy.3A8	VB17	Hy.1H3	VB14	Fn.1M7	VB4
Hy.3A10	VB17	Hy.2B2	VB14	Fn.3E17	VB3,VB5
Hy.3B9	VB17	Hy.2H9	VB14	Fn.1E6	VB6,VB8
Hy.3C7	VB17			Fn.1G6	VB17
Hy.3G10	VB17	Patient 2 (DR2,DRw11)		Patient 5 (DR3,DR4)	
Hy.3F6	VB17	Cy.2H11	VB1,VB7	Tw.1B11	VB12
Hy.3F7	VB17	Cy.3D2	VB1,VB7	Tw.2F3	VB12,VB17
Hy.3F10	VB17	Cy.2C6	VB2	Tw.E10	VB17
Hy.1A8	VB17	Cy.2G5	VB17	Tw.2E2	VB14

TABLE 3 (Cont'd)

CONTROLS

CELL LINE	TCR VB	CELL LINE	TCR VB	CELL LINE	TCR VB
Control 1 (DR2,DR4)		Control 2 (DR2)		Control 4 (DR7, DRw11)	
Rt.1A9	VB17	Hr.1B7	VB12	An.3E1	VB1,VB8
Rt.3C1	VB17	Hr.1C9	VB5	An.3H3	VB8
Rt.3G11	VB17	Control 3 (DR2)		An.3C12	VB2
Rt.3A3	VB17, VB14	Md.2A4	VB6,VB8	Control 5 (DR1,DR9)	
Rt.3F1	VB17, VB14	Md.2F1	VB8,VB18	Cr.1B12	VB17,VB12

MBP PEPTIDE 143-168 REACTIVE T-CELL LINES

MULTIPLE SCLEROSIS

CELL LINE	TCR VB	CELL LINE	TCR VB
Patient 2 (DR2, DRw11)		Patient 3 (DR2, DR4)	
Cy.1E6	VB14	Ns.2D6	VB3
Cy.2B12	VB14	Patient 4 (DR2, DR7)	
Cy.2E2	VB14	Fn.1H5	VB4
Cy.3G10	VB14	Fn.2A10	VB4

Cy.3H10	VB14,VB8	Fn.2A5	VB2
Cy.4C10	VB14,VB17	<u>Patient 5 (DR3, DR4)</u>	
Cy.1C12	VB12	Tw.2C9	VB12
Cy.1E9	VB7		
Cy.3F9	VB1		

CONTROLS

CELL LINE	TCR VB	CELL LINE	TCR VB
<u>Control 3 (DR2)</u>		<u>Control 6 (DR1, DR7)</u>	
Hr.2E10	VB3, VB5	Bn.2G1	VB12
Hr.3E9	VB7	Bn.3D6	VB12
		Bn.3C10	VB5,VB8

Fifty-one of these lines reacted with MBP residues 84-102, while fourteen T-cell lines were specific for MBP residues 143-168. Thirty-one MBP T-cell lines reactive to MBP amino acid residues 84-102 were analyzed from MS patient Hy (patient 1, Table 3). Twenty-three of these T-cell lines (74%) were found to use the VB17 gene segment, while eight other cell lines were restricted by either VB2, VB7 or VB14 gene segments. These results indicate that VB17 is the major recognition element in T-cell lines from this MS patient reactive with MBP residues 84-102. VB17 usage was also found among 6/20 T-cell lines examined from four other patients (patients 2-5, Table 3). The second TCR VB that was used by T-cell lines among these four patients was VB12 which was found in 7/20 T-cell lines reactive with MBP residues 84-102 (Table 3, Figure 1). This VB happens to be homologous to the mouse VB8.2 which is the predominant TCR used among encephalitogenic T-cells in mice and rats (Burns, F.R. et al., <u>J</u>. <u>Exp</u>. <u>Med</u>. <u>169</u>: 27, 1989).

MS patient Cy expressed both the DR2 and DRw11 antigens and thus had T-cells that recognized either the immunodominant MBP region (84-102 residues) or the MBP 143-168 residues. This provided the opportunity to compare TCR VB usage among T-cells reacting to different MBP determinants (Figure 1). Of seven lines proliferating to MBP residues 84-102, three expressed VB12 and one expressed VB17 (Table 3). In contrast, 6/9 T-cell lines recognizing the MBP residues 143-168 used VB14 and only one line

each used the TCR VB12 and VB17 TCR genes (Table 3). Southern blot analysis of five T-cell lines reactive with MBP residues 84-102 (VB12: Cy.2C2, Cy.3F6) or MBP residues 143-168 (VB14:Cy.1E6, Cy.2B12, Cy.2E2) are shown in Figure 1.

While VB12/VB13 is relatively common among normal peripheral blood T-cells (approximately 18%), VB17 is significantly less frequent (approximately 3%), as assessed by quantitative PCR. In contrast, VB17 was found in 34/63 (53.9%) of T-cell lines reactive with MBP residues 84-102, while it was only present in 3/32 (9.4%) of TCR VB genes in random mitogen derived T-cell clones obtained by single-cell cloning from a normal individual (Moretta, A. et al., J. Epp. Med. 157: 743, 1983; Hafler, D. A., et al., J. Exp. Med. 167: 1313, 1988). These data indicate that the VB17 TCR is selectively involved in the recognition of the immunodominant MBP 84-102 region.

In order to show that the TCR gene segment identified by PCR was the VB encoding gene used to recognize the MBP peptide, two VB17 positive T-cell lines (Hy.2H9 and Hy.2G5) were cloned by limiting dilution (Moretta, supra.). 11/11 individual clones established from these two cell lines, which were reactive with both MBP and MBP residues 84-102, were VB17+. Three of these clones were further analyzed using the complete panel of VB primers and were all found to be negative for the other VB segments.

The VB sequences of four T-cell lines from patient Hy were found to be 100% homologous to the published VB17 sequence (as disclosed in Kimura, N., et al., Eur. J. Immunol. 17: 375, 1987). This sequence analysis confirms that specific VB segments were indeed amplified using this approach. Analysis of the VDJ (diversity-junctional) sequence indicated that all four of these T-cells used the same junctional JB2.1 segment and that 3/4 of them had the same VDJ sequence (Table 2). To determine how frequently the JB2.1 gene segment was used by VB17+ T-cells, the DNAs from 20 cell lines from MS patient Hy were amplified using the VB17 primer combination with a CB primer or a JB2.1 primer (Figure 2). All of these lines were found to be positive for VB17 as well as JB2.1 gene segments, while the negative controls

(RNA extracted from all cell lines and not converted to cDNA, and reagents used for cDNA synthesis and amplification) were negative by PCR and Southern blotting. These data show a strong selection for the VB17-JB2.1 sequence elements in with MBP residues 84-102 reactive T-cell lines derived from patient Hy.

Two other T-cell lines using the VB17 TCR identified by PCR analysis and recognizing MBP residues 84-102 from MS patients Fn and Ns were sequenced and compared to sequences of TCR VB from MS patient Hy (Table 3). While the VB17 gene segment sequence was identical among T-cells reactive MBP residues 84-102 from the three patients, different JB sequence elements were found. Three results show a shared VB gene usage in T-cells recognizing an immunodominant MBP peptide between different individuals. In contrast, shared JB gene segment usage was found among T-cells derived from the same individual but not between different individuals.

Four of the five patients studied were positive for the disease-associated DR2 allele, while patient Tw was HLA-DR3, DR4. Nevertheless, three VB12/VB17 restricted cell lines were present among four lines analyzed from this MS patient (Table 3), indicating that shared MHC Class II antigens may not be mandatory for shared TCR VB gene usage with respect to recognition of MBP peptide 84-102.

EXAMPLE 3: IDENTIFICATION OF THE MAJOR IMMUNODOMINANT REGION OF HUMAN MBP

A rapid T-cell cloning technique was used to examine whether there were immunodominant epitopes on human MBP reactive with Class II MHC phenotypes and the frequency of such reactivity. A total of 15,824 short term T-cell lines were generated from 51 subjects by culturing peripheral blood mononuclear cells (PMN) with purified MBP (obtained as in Example 1 above) followed 3 days later, and then every 3-4 days, by the addition of Interleukin-2 (IL-2) and Interleukin-4 (IL-4) (Genzyme, Boston, MA). On Day 13 of culture, an aliquot from each line was tested for reactivity to MBP. Lines reactive to MBP were then tested for reactivity to overlapping oligopeptide 20-mers encompassing the human MBP sequence as shown in Table 1 above. For MHC restric-

tion experiments, lines reactive to an MBP peptide were restimulated for two more cycles, first with MBP and then with the specific MBP fragment recognized by that line. In a subgroup of patients, the frequency of T-cells recognizing proteolipid protein (PLP), another major encephalitogenic central nervous system antigen, was investigated.

MBP and PLP frequency analysis was performed on patients with definite, relapsing-remitting MS (as diagnosed by Magnetic Resonance Imaging -- "MRI" -- and clinical examination), as well as on subjects with other neurologic diseases and normal subjects (all age and sex matched to the MS patients).

The results are shown in Table 3A below.

TABLE 3A

	AGE	SEX(%) (M/F)	MHC(%) DR2	DR4	DRw11	DQw1	#Ag REACTIVE LINES/ TOTAL # LINES MBP	PLP	MEAN FREQUENCY OF Ag REACTIVE LINES (%) MBP	PLP
MULTIPLE SCLEROSIS (n=23)	34.2+1.4	35/65	60.9	26.1	13.0	78.2	554/7746	20/432	7.18±2.38	3.34±1.56
OTHER NEUROLOGIC DISEASE (n=10)	38.7±3.2	43/57	14.3	0.0	42.9	85.7	118/2880	3/384	4.10±1.04	0.90±0.62
NORMAL (n=6)	30.3±1.5	50/50	16.7	0.0	50.0	66.6	73/1742	ND	4.70±1.58	ND
DR2+ CONTROLS (n=6)	32.0±2.9	50/50	100	16.7	0.0	100	53/1728	ND	3.08±2.06	ND

Patients with MS were caucasian and had well- characterized relapsing remitting disease with at least two exacerbations within the previous 24 months and positive lesions on Magnetic Resonance Imaging (MRI) at the time of blood drawing. Subjects with other central nervous system diseases had the following diagnoses: 1-3 weeks after either cerebrovascular accident [4] or brain trauma with CNS hemorrhage [4]; metastatic brain tumor [2]. The total number of T-cell lines reactive with either MBP or PLP and the total number of T-cell lines generated are shown in Table 3A ("Ag" means "antigen"). In addition, the

frequencies of MBP- and PLP- reactive lines were calculated separately for each subject by dividing the number of MBP-reactive lines by the total number of lines generated and the mean value $\pm$ SEM are given.

While the frequency of MBP reactive lines was slightly higher in subjects with MS as compared to the other subjects, this was not statistically significant. There was more reactivity to PLP in patients with MS as compared to subjects with other neurologic diseases, but this did also not reach statistical significance.

Of a total of 302 cell lines from patients with MS that could be expanded and confirmed to react with MBP on repeated analysis, 140 (46.4%) reacted with MBP residues 84-102. In the control groups, 11 of a total of 100 MBP reactive T-cell lines (11.0%) recognized this MBP peptide. The actual frequency of T-cells derived from the peripheral blood that reacted with each MBP peptide for each individual subject was calculated. The mean values for patients with MS and the control subjects are shown in the next-to-rightmost column of Table 3A.

50,000 T-line cells were plated in triplicate with 50,000 irradiated APC, MNC (mononuclear cells) (Hafler, D. A., et al., J. Exp. Med. 167: 1313, 1988) for 72 hours in round bottom 96-well microtiter plates and wells were pulsed with [^{3}H]-thymidine for the last 18 hours of culture. APC MNC were either cultured alone, pulsed with 100 micrograms/ml of synthetic MBP peptide 84-102, (determined to be the optimal concentration of peptide to induce proliferation), or pulsed with 100 micrograms/ml of MBP. The average counts per minute (CPM) values for triplicate wells are shown in Table 4. DR and DQw haplotypes are given and haplotypes common with the patient (top line), who was positive for DR2, DR7, DQw1, DQw3, are underlined.

Proliferation of T-cell lines using a panel of different mononuclear cells (MNC) as antigen presenting cells (APC) are shown. Five T-cell lines reactive to MBP amino acid residues 84-102 from subject Hy were expanded by repeated cycles of stimulation with autologous irradiated MNC, pulsed with synthetic

MBP peptide 84-102 and examined for recognition of this region of MBP.

For these studies, the panel of five T cell lines reactive with MPB residues 84-102 were plated with autoautologous APC MNC, as above, in the presence of monoclonal antibodies (mAbs) (final concentration of 1:100) recognizing different MHC Class II gene products. (The nomenclature used for the antibodies is from the Tenth International Histocompatibility Workshop; their specificity is also given). The results are set forth in Table 5 below.

TABLE 4

T cell lines from patient Hy

MHC Phenotype of APC		1A8			2C9			2E11			2H9			3A10		
DR	DQw	APC	MBP	peptide 84-102	APC	MBP	peptide 84-102	APC	MBP	peptide 84-102	APC	MBP	peptide 84-102	APC	MBP	peptide 84-102
2.7	1.3	32	21,192	10,747	83	3,263	14,991	148	18,593	30,368	169	2,797	10,444	139	6,887	24,411
2	1	83	56	32	82	78	112	217	52,939	49,399	636	327	658	23	28	26
4.7	2.3	32	26	53	45	55	142	37	167	81	226	258	263	306	719	915
3	2	46	32	52	43	44	110	101	98	349	769	402	1,973	23	31	100
3.10	1.2	35	30,737	49,144	158	25	80	36	58	42	49	54	46	42	22,823	31,121
2.7	1.2	38	40	47	43	39	43	78	53,441	32,357	261	190	289	33	19	36
7.w11	2.7	44	39	54	57	124	259	34	25	33	51	58	97	967	1,214	2,744

The frequency of peptide specific cell lines from normal subjects and other neurologic disease controls were virtually identical and thus combined for analysis. The mean frequency of T-cell lines from subjects with MS that were selectively reactive to MBP residues 84-102 was higher as compared with controls (Figure 3). Significant but less striking increases in reactivity to MBP residues 61-82 and 124-142 were also observed in MS patients, while both MS and control subjects showed high frequencies of T-cell lines reactive with MBP residues 143-168. The DR2, DQw1 haplotype was very infrequent in the control subjects and more common in patients with MS (Table 4). An association was observed between the DR2 phenotype and both the proportion or the frequency of T-cell lines reactive to MBP residues 84-102 (Figure 4).

To determine if T-cell reactivity to MBP residues 84-102 was associated with DR2, DQw1 expression in non-MS subjects, an additional 6 normal subjects with DR2, DQw1 phenotype were investigated. The results are shown in Figure 4.

A DR2 association was also observed among controls in terms of the proportion of T-cell lines reactive with MBP residues 84-102 (DR2+ controls, 31.0±10-.8%; DR2-, 10.1±0.4%), though the total frequency of lines reactive with this region of MBP was less than that in patients with MS (Figure 4). Though DQw1 is in linkage dissociation with DR2 as well as with DR1 and DRw10, independent analysis of peptide reactivity revealed no association with DQw1 phenotype expression.

The DRw11 phenotype was more common in controls than in subjects with MS (Table 3A). DRw11 was positively associated with the frequency of lines reactive to MBP residues 142-168 in patients with MS and controls, but not with the frequency of lines reactive with MBP residues 84-102 (Figure 2). Reactivity to MBP residues 31-50, which was predominantly observed in control subjects, was associated with DRw11. Other MHC associations were not observed.

The MHC association with residues of the T-cell lines reactive with an immunodominant MBP epitope was determined. The results are set forth in Table 5 below. More specifically, it

was determined whether the MHC haplotypes were used to present antigen in the T-cell lines reactive with an immunodominant MBP epitope.

<u>Table 5</u>

mAb specificity		T-cell lines from patient Hy 1A8	2C9	2E11	2H9	3A10
	APC alone	32	83	39	50	139
	no mAb	10,747	14,991	3,325	8,659	24,411
	control mAb	11,375	15,322	4,131	8,156	27,363
anti-DR	PL8	11,051	41	31	142	25,016
	L.243	16,792	586	22	36	21,148
	65P4.1	19,119	405	46	92	26,412
anti-DQ	1A3	4,851	11,444	2,102	5,446	15,714
	Tu22	1,189	13,442	1,073	7,661	13,488
	Leu10	1,128	14,924	2,255	7,678	13,090
anti-DP	B7121	7,917	15,922	2,337	6,689	23,452
anti-DR+DP	Tu35	13,606	75	21	42	27,104

Monoclonal antibody blocking studies of five T-cell lines reactive with MBP residues 84-102 suggested that both DR and DQ molecules could function as restricting elements. Among clones blocked by anti-DR mAb, clone 2E11 proliferated in response to MBP residues 84-102 with the panel of DR2+ APC while 2C9 and 2H9 proliferated only with autologous APC (Table 5). The recognition of peptide by clones 1A8 and 3A10, which were partially blocked by anti-DQ mAbs was restricted to APC from the responder and one of two APC donor subjects expressing DQw1.

To investigate further the relationship between MHC expression and frequency of T-cell reactivity to immunodominant MBP epitopes, a family with one afflicted sibling expressing both DR2 and DRw11 phenotypes was studied.

The family members of an MS patient expressing the DR2, DQw1; DRw11, DRw52, DQw1 Class II MHC haplotypes were examined for the frequency of T-cell lines reactive with MBP residues 84-102 and 143-168.

A total of 1,728 individual T-cell lines were generated from both parents and 4 siblings and the number of lines reactive with either MBP peptide 84-102 or 143-168 were determined.

$2x10^5$ MNC in each of 288 wells (three 96 well round bottom plates) were cultured with MBP (10 micrograms/ml) as outlined above for each subject. On day 16, each T-cell line was analyzed for reactivity to synthetic peptides corresponding to the MBP residues 84-102 and 143-168. The number of lines reactive with each peptide (stimulation index SI>3, delta CPM>500) generated per subject are shown. The actual stimulation indices were generally >20. P1 and P2=parents; S1-S3=siblings. The results are set forth in Table 6 below.

TABLE 6

PATIENT	P1	P2	S1	S2	S3
	DR4		DR4	DR4	
DR2	DRw53	DR2	DRw53	DRw53	DR2
DQw1	DQw3	DQw1	DQw3	DQw3	DQw1
DRw11	DRw11	DRw6	DRw6	DRw6	DRw4
DRw52	DRw52	DRw52	DRw52	DRw52	DRw53
DQw1	DQw1	DQw1	DQw1	DQw1	DQw3

MBP peptide	PATIENT	P1	P2	S1	S2	S3
84-102	49	1	4	6	1	7
143-168	41	14	0	3	2	2

The DR2+, DRw11+ patient had a high frequency of T-cell lines reactive to both MBP residues 84-102 and 143-168. The DRw11+ parent preferentially recognized MBP residues 143-168, while the DR2+ parent preferentially recognized MBP residues 84-102. The frequency of MBP peptide reactive lines, however, was lower than that of the patient. One sibling was DR2+ and preferentially recognized MBP residues 84-102. Of two HLA identical siblings with DR4, DQw3/DRw6, DQw1, one reacted to MBP peptide 84-102 whereas the other did not. Although DQw1 may restrict recognition of MBP residues 84-102, other factors such as inherited TCR polymorphism may have influenced T-cell reactivity to the MBP autoantigen in one of the DR4, DQw3/DRw6, DQw1 siblings. This family linkage analysis suggested that optimum recognition of immunodominant MBP epitopes requires specific Class II MHC alleles both in patients with MS and in controls. In total, these studies indicate that although control subjects expressing DR2 appear to preferentially recognize the same MBP determinant as compared to DR2+ MS patients, their frequency in the blood is less than that of patients with MS.

EXAMPLE 4: SEQUENCING OF VB17 TCR

The T-cell receptor VB17+ PCR products from six cloned T-cell lines were sequenced by the dideoxy method as described reactive with MBP residues 84-102 (Patients Hy, Fr and Ns) in

Example 1. The DNAs were amplified using PCR primers for the VB17-leader sequence and the TCR CB region described above in Example 1. The amplified DNA was cloned into M13 and sequenced using the well-known dideoxy method (3 M13 plaques per T-cell line). The results are set forth in Table 7 below.

TABLE 7

	VB	DB	JB	
Hy.1A8	TyrLeuCysAlaSerSer TATCTCTGTRGCCAGTAGT	ThrAspTrpSer ACTGACTGGAGC	SerTyrAsnGluGlnPhe TCCTACAATGAGCAGTTC	VB17-JB2.1
Hy.2C9	TyrLeuCysAlaSerSer TATCTCTGTGCCAGTAGT	ThrAspTrpSer ACTGACTGGAGC	SerTyrAsnGluGlnPhe TCCTACAATGAGCAGTTC	VB17-JB2.1
Hy.3A10	TyrLeuCysAlaSerSer TATCTCTGTGCCAGTAGT	ThrAspTrpSer ACTGACTGGAGC	SerTyrAspGluGlnPhe TCCTACAATGAGCAGTTC	VB17-JB2.1
Hy.2C8	TyrLeuCysAlaSerArg TATCTCTGTGCCAGTAGG	ThrSerGly ACTAGCCGGC	SerTyrAsnGluGlnPhe TCCTACAACGAGCAGTTC	VB17-JB2.1
Fn.1G6	TyrLeuCysAlaSerSer TATCTCTGTGCCAGTAGT	IleProPro ATCCCTCCA	SerTyrGluGlnTyrPhe TCCTACGAGCAGTACTTC	VB17-JB2.7
Ns.1G11	TyrLeuCysAlaSerSer TATCTETGTGCCAGTAGT	AlaAspArg GEGGACAGG	AspGlnProGlnHisPhe GATCAGCCCCAGCATTTT	VB17-JB1.5

It should be noted above that the VB17 sequence of all 4 T-cell lines established from MS patient Hy were 100% homologous to the published VB17 sequence.

The following is a concordance between the 3-letter and the 1-letter codes for aminoacids. It is provided for convenience.

Aspartic acid
(Asp, D)

Glutamic acid
(Glu, E)

Lysine
(Lys, K)

Arginine

(Arg, R)

Histidine
(His, H)

Tyrosine
(Tyr, Y)

Cysteine
(Cys, C)

Asparagine
(Asn, N)

Glutamine
(Gln, Q)

Serine
(Ser, S)

Threonine
(Thr, T)

Glycine
(Gly, G)

Alanine
(Ala, A)

Valine
(Val, V)

Leucine
(Leu, L)

Isoleucine
(Ile, I)

Methionine
(Met, M)

Proline
(Pro, P)

Phenylalanine
(Phe, F)

Tryptophan
(Trp, W)

WHAT IS CLAIMED

1. A human immunogen comprising a purified peptide containing at least a portion of the T-cell receptor for an antigen that activates human immune response against myelin basic protein.

2. A composition comprising the immunogen of claim 1 in a pharmaceutical dosage form suitable for administration to humans.

3. A mammalian cell comprising an attenuated human T-cell containing a T-cell receptor encoded by a member selected from the group consisting of VB-12 and VB-17.

4. A mammalian cell comprising an attenuated human T-cell containing a T-cell receptor for an antigen that activates the human immune response against myelin basic protein.

5. A peptide comprising the amino acid sequence of all or a portion of human VB12 or VB17, said peptide having the property of suppressing one or more multiple sclerosis symptoms when administered to a human presenting with said symptoms.

6. A peptide comprising all or a portion of the amino acid sequence of at least one of human VB12, human VB17 and analogs thereof, said peptide having the property of suppressing or attenuating symptoms of multiple sclerosis in a human.

7. A peptide comprising all or a portion of the amino acid sequence of at least one of human VB12, human VB17 and analogs thereof, said peptide having the property of suppressing or eliminating T-cells in a human which induce multiple sclerosis symptoms.

8. A peptide comprising all or a portion of the amino acid sequence of at least one of human VB12, human VB17 and analogs thereof, said peptide having the property of inhibiting recognition of an immunodominant domain of human myelin basic protein by human T-cells.

9. A peptide comprising an aminoacid sequence selected from the group consisting of IYYSQIVNDFQKGDIAEGYS

Asp-Thr-Asp-Lys-Gly-Glu-Val-Tyr-Asp-Gly

and

Phe-Gln-Lys-Gly-Asp-Ile-Ala-Glu-Gly-Tyr.

10. A composition of matter comprising a peptide according to any one of claims 5-9, said peptide being bound to or embedded within a larger complex or molecule.

11. The composition of claim 10, said composition having the amino acid sequence of human antigen recognition receptor on the surface of human T-cells.

12. The composition of claim 11, said receptor recognizing an immunodominant epitope of human myelin basic protein.

13. The composition of claim 10, said composition comprising said peptide covalently bound to an adjuvant.

14. A vaccine against the symptoms of multiple sclerosis, comprising, in an amount effective to suppress or attenuate one or more multiple sclerosis symptoms upon administration to a human, isolated, attenuated VB17+ or VB12+ human T-cells from a human subject diagnosed with multiple sclerosis.

15. A method for treating or preventing a disease having the symptoms of multiple sclerosis comprising administering to a mammal in need of such treatment an agent comprising a peptide having all or a portion of the amino acid sequence of the human T-cell antigen recognition receptor in an amount effective to treat or prevent said symptoms.

16. A kit for diagnosing a mammal for multiple sclerosis comprising: nucleic acid having a nucleotide sequence corresponding to all or a portion of the nucleotide sequence encoding at least one of human VB12 and VB17 and sequences which hybridize therewith under stringent hybridization conditions.

17. The kit of claim 16 wherein said nucleotide sequence comprises a member selected from the group consisting of

5'-GAT ACT GAC AAA GGA GAA GTC TCA GAT GGC-3';

5'-TTT CAG AAA GGA GAT ATA GCT GAA GGG TAC-3';

5'-ATC TAC TAC TCA CAG ATA GTA AAT GAC TTT CAG AAA GGA GAT ATA GCT GAA GGG TAC AGC-3' and sequences complementary thereto.

18. The peptide of claim 8 wherein said sequence is different from those of rodent VB12 and VB17.

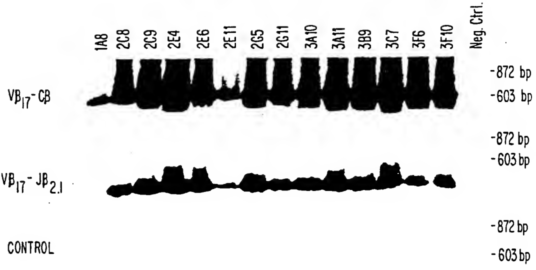
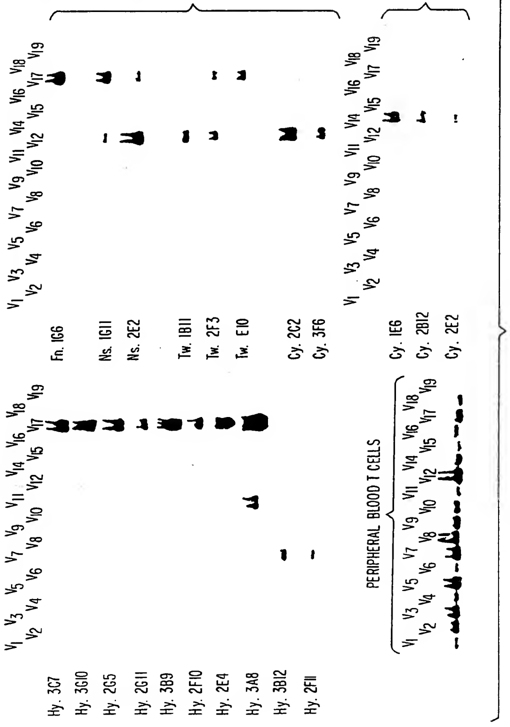

1A8
2C8
2C9
2E4
2E6
2E11
2G5
2G11
3A10
3A11
3B9
3C7
3F6
3F10
Neg. Ctrl.
VB17-CB
-872 bp
-603 bp
VB17-JB2.1
-872 bp
-603 bp
CONTROL
-872 bp
-603 bp

FIG. 1

FIG. 2


84 - 102 REACTIVE LINES
143 - 163 REACTIVE LINES
V1 V2 V3 V4 V5 V6 V7 V8 V9 V10 V11 V12 V14 V15 V16 V17 V18 V19
Fn. 1G6
Ns. 1G11
Ns. 2E2
Tw. 1B11
Tw. 2F3
Tw. E10
Cy. 2C2
Cy. 3F6
Cy. 1E6
Cy. 2B12
Cy. 2E2
Hy. 3C7
Hy. 3G10
Hy. 2G5
Hy. 2G11
Hy. 3B9
Hy. 2F10
Hy. 2E4
Hy. 3A8
Hy. 3B12
Hy. 2F11
PERIPHERAL BLOOD T CELLS

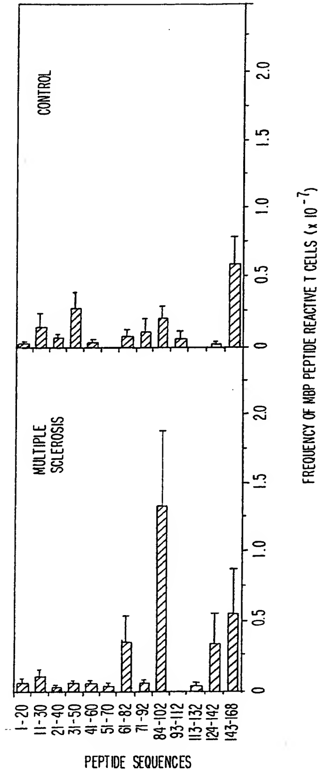

CONTROL
MULTIPLE SCLEROSIS
0
0.5
1.0
1.5
2.0
FREQUENCY OF MBP PEPTIDE REACTIVE T CELLS (x 10⁻⁷)
1-20
11-30
21-40
31-50
41-60
51-70
61-82
71-92
84-102
93-112
113-132
124-142
143-168
PEPTIDE SEQUENCES

FIG. 3

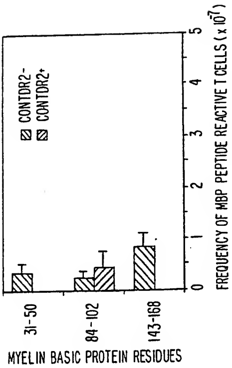


FIG. 4B

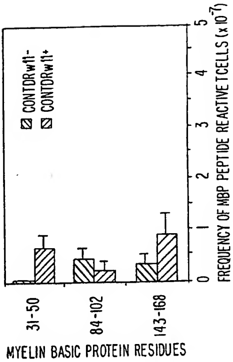


FIG. 4D

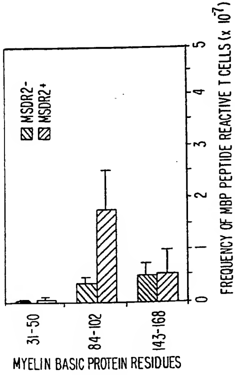


FIG. 4A

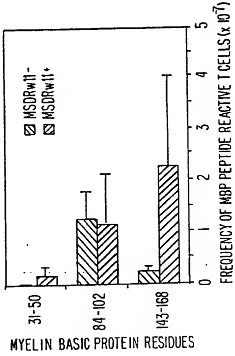


FIG. 4C

SUBSTITUTE SHEET

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US91/02218

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC
IPC(5): A61K 37/02; C12N 5/06, 5/08; C12Q 1/68
US CL : 530/326; 514/13; 435/240.1,6

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S. CL.:	530/326; 514/13; 435/240.1,6

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [*]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X Y	Nature, Vol. 341, issued 12 October 1989. Vandenbark et al. "Immunization with a synthetic T-cell receptor V-region peptide protects against experimental autoimmune encephalomyelitis" pages 541-544, see page 542.	1-4 5-18
X,P Y	Science, Vol. 248, issued 1990, Wucherpfennig et al., "Shared human T-cell Receptor V-Beta usage to immunodominant regios of myelin basic protein", see page 4958, abstract no. 90:334456.	1-12 13-18
X,P	J. Exp. Med., Vol. 171, issued June 1990, Zaller et al., "Prevention and Treatment of Murine Experimental Allergic Encephalomyelitis with T-cell Receptor V.B. Specific Antibodies", pages 1943-1955. See entire document.	3 and 4
Y,P	Journal of Immunology, Vol. 144, No. 12, issued 15 June 1990, Hashim et al., "Antibodies Specific for VB8 Receptor Peptide Suppress Experimental Autoimmune Encephalomyelitis", pages 4621-4627, see entire document.	1-18

* Special categories of cited documents: [10]
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
01 JULY 1991	01 August 1991
International Searching Authority	**Signature of Authorized Officer**
ISA/US	Iila Feisee

Form PCT/ISA/210 (second sheet) (Rev.11-87)

International Application No. PCT/US91/02218

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers ...., because they relate to subject matter [12] not required to be searched by this Authority, namely:

2. ☐ Claim numbers ...., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [13], specifically:

3. ☐ Claim numbers ___________, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.